International Research Journal of Biological Sciences ________________________________E-ISSN 2278-3202
Vol. 5(11), 27-32, November (2016) Int. Res. J. Biological Sci.

Review Paper

Amylases: A Note on Current Applications

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Available online at: www.isca.in, www.isca.me
Received 4th October 2016, revised 29th October 2016, accepted 7th November 2016

Abstract

Enzymes have been used successfully for different types of industries and microorganisms have proved their recognition in the production of enzymes invaluable for industry. Amylase is a highly efficient commercial biocatalyst with an extensive range of utilizations from food to non-food industries. The hydrolytic activities of amylases on glycosidic bonds in starch have successfully replaced chemical processing of starch in different industries due to cost effectiveness and technical advantages. Production of glucose/fructose syrups and most widely used biofuel results from the catalytic activity of amylases. Amylases are used for a variety of applications in different enterprises including food, paper, detergent and textile industry. Additionally, amylases may also have potential application in pharmaceutical and fine chemical industries.

Keywords: Amylase, Starch, Enzyme, Application, Industry.

Introduction

Enzymes, a vital component of the biological system, have been exploited for alcohol as well as other beverages production since ancient times[1]. Microorganisms have successfully proved their recognition as a rich source of useful enzymes. Several enzymes including amylases, cellulases, lipases, proteases, xylanases, etc. are currently available for a variety of commercial applications. Among these enzymes, α-amylase is one of the highly versatile enzyme in commercial areas owing to high abundance of starch on the Earth, with numerous uses including production of sugar syrups, biofuel and pharmaceutically important cyclodextrin[2-4].

Interestingly, the first commercially produced enzyme of a microbial source was an amylase of fungal origin in 1894 and, it used as a therapeutic aid to cure digestive disorder[4-6]. Amylases are a group of commercial enzymes that share nearly 25% of the global enzyme production[7,8]. They are among the most important enzymes and are utilized for a variety of applications in food and non-food industries. Their applications have also been expanded to other fields including clinical and analytical chemistry.

Amylases, hydrolytic enzymes, catalyze the hydrolysis of starch into low molecular weight sugar molecules. In the nature, starch is the most abundant polysaccharide food store after cellulose and the primary accessible source of carbon and energy on the Earth[9,10]. It is synthesized by plants and utilized in food, textile, paper, alcohol, pharmaceutical industries[10]. Starch is deposited in plant cells in the form of granules as reserve material, which is a primary component of bread and other food products and, used as gelling agent, emulsion stabilizer, thickener, fat alternative and water binder for many food products[11].

Amylases are ubiquitous and distributed throughout the plants, animals and microbes. However, microorganisms producing amylases have successfully replaced chemical processing methodology in different industries due to cost effectiveness and technical advantages[12,13]. Currently, with expanding application areas, the research is primarily focused on the development of new amylases with high pH and temperature stability to achieve enhanced rate of catalysis, improved gelatinization of starch, decreased media viscosity and diminished possibility of microbial contamination[3]. The ubiquitous occurrence, easy production, and a wide range of uses make α-amylase a commercially valuable biocatalyst[14]. In this communication, we are presenting an overview of applications of amylases from microbial sources.

Classification

Amylases can be classified into two groups, endo- and exo-amylases on the basis of their mode of action. The endo-amylases catalyze random hydrolysis of α-1,4 glycosidic bonds present in the structural components i.e. amylose or amylopectin chain of starch. This catalytic activity results in the formation of linear and branched oligosaccharides of different chain lengths. The exo-amylases hydrolyze from the non-reducing end, successively resulting formation of short end products. Exo-amylase such as β-amylase hydrolyze α-1,4 glycosidic linkage and glucoamylase catalyze cleavage of α-1,4 and α-1,6 glycosidic bonds[15-17].

Table-1
Classification of amylases[13,18]

Enzyme	Glycosidic bond specificity	Mode of action	Product
α-amylase (1,4-α-D-glucan glucanohydrolase)	α-(1-4)-glucosyl	Endo oligosaccharides	Linear and branched
β-amylase (1,4-α-D-glucan maltohydrolase)	α-(1-4)-glucosyl	Exo Dextrins	Maltose and limit
Amyloglucosidase (Exo-1,4-α-glucosidase; glucoamylase)	α-(1-4)-glucosyl and Glucose α-(1-6)-glucosyl	Exo/Endo	Glucose

α-Amylase (EC 3.2.1.1), calcium metalloenzyme, is also known as glycogenase or 1,4-α-D-glucan glucanohydrolase[17,19]. They hydrolyze α-1, 4 bonds arbitrarily and cleave long chain sugars to yield maltotriose and maltose from amylose and maltose, glucose and limit dextrin from amylopectin[20]. α-Amylases are derived from plants, animals, bacteria (Bacillus) and fungi (ascomycestes and basidomycetes)[17].

β-amylase (EC 3.2.1.2) hydrolyze α-1,4 glycosidic linkages from non reducing ends and cannot bypass α-1,6 linkages unlike α-amylase. It is produced by plants, bacteria and fungi[21]. Though, β-amylase is absent in animal tissues, it may be present in the microbes that live in the digestive tracts. β-amylase hydrolyze starch into maltose in ripened fruits resulting in sweet flavour[17,22].

Both forms, α- and β-amylase are present in seeds. β-amylase is present in an inactive form before germination whereas the α-amylase is appear on germination.

γ-Amylase (EC 3.2.1.3) catalyze hydrolysis of α-1,4 and α-1,6 glycosidic linkages. It is alternatively called as glucoamylase, exo-1,4-α-glucosidase, amyloglucosidase, glucan 1,4-α-glucosidase, 1,4-α-D-glucan glucohydrolase or lysosomal α-glucosidase. Unlike α- and β-amylases, this amylase is optimally active in acidic environments[23,24].

Commercial Applications

The global demand of enzymes, for a wide variety of applications, is significant. Amylases have extensive applications in starch based food and non-food industries (Table-2).

Starch processing industry: Starch, the second main polysaccharide food store in the nature, is the primary constituent of majority of staple foods and used in various food and non-food industries. Starch is produced by plants through photosynthesis and it is an easily accessible source of carbon on the Earth. Starch is utilized in many industries including food, alcohol, paper, textiles etc. Processing of starch is necessary for most of the applications in different industries. Chemical processing of starch has disadvantages such as requirement of high temperature, low pH, lower yield of glucose, unpleasant taste of compounds and synthesis of undesired colour whereas enzyme mediated hydrolysis has the capabilities overcoming these unfavorable features[10,25]. α-Amylase mediated starch hydrolysis is used in the production of glucose and fructose syrups[26]. Bioconversion of starch to syrups, most widespread application of amylase in the starch industry, include dissolution of starch granules to form a viscous suspension, partial hydrolysis to reduce viscosity and further hydrolysis to produce glucose and fructose syrups as end products [2,27]. Thermostable α-amylases, particularly from *Bacillus* strains, are exploited for the processing of starch as liquefaction and saccharification is performed at high temperature[28].

Ethanol has been considered for use as a fuel since early days of automobile. The conversion of starch to ethanol involve amylase mediated liquefaction & saccharification of starch to produce sugar, followed by sugar fermentation using yeast *Saccharomyces cerevisiae* for ethanol production[2,28,29]. For ethanol production, starch is primarily used as a substrate due to cost effectiveness and ease of availability in maximum areas globally[30]. The α-amlyases from thermostable *Bacillus licheniformis* and engineered *Escherichia coli* and *B. subtilis* are utilized for the hydrolysis of starch suspensions[31].

Table-2
Use of amylases in different industries[1,2,7]

Industry	Applications
Food	Starch liquefaction and saccharification; manufacturing of corn syrups; anti-staling in baking; Enhance shelf life of breads; reduction of chill haze formation in beverages
Detergent	Removal of starch based stains
Paper	Reduction of viscosity, Deinking, drainage improvement
Textile	Desizing, Warp sizing of textiles fibers
Biofuel	Ethanol production
Pharmaceutical	Digestive aid
Bioremediation	Bioremediation of vegetables wastes
Leather	Fiber splitting

Food industry

The advent of modern biotechnology has brought many changes in the food industry. The application of enzymes in food industry is diverse and most often, they are applied as processing agents. Amylases are widely utilized in processed food industry including baking, beverages, starch syrups etc. Production of bread is one of the most common food processing technique globally[32]. The application of enzymes in the manufacturing of bread demonstrates their significance in quality control and efficiency of production. Amylase, solely or in a mixture of enzymes, is added to the flour to prolong freshness and shelf life of the baking products[1]. Amylases are added to the dough in baking products, such as bread to hydrolyze flour polysaccharide into dextrins, which are fermented by the yeast[14]. The addition of α-amylase improve the rate of fermentation and reduces the viscosity of flour dough, and consequently in the improvement of volume and texture[29]. Besides, amylases mediated hydrolysis produces extra sugar and enhance the qualities including taste of the baking products. Another important use of amylase in baking is anti-staling by mild hydrolysis of starch polymers, to prevent polymer crystallization and thereby hardening of bread[2]. This application of amylase prolongs crumb firmness, softness retention and shelf life of baked goods. The α-amylase and glucanase from *Bacillus amyloilquifaciens* are utilized to enhance enzyme mediated liquefaction of the malt and glucanase causes reduction of viscosity of wort to improve beer filtration[33].

Amylases are utilized in the clarification of juices to maximize the production of clear juice[34,35]. Amylases derived from microorganisms, may also be utilized in the alcoholic beverages to minimize or eliminate turbidities by hydrolyzing starch prior to fermentation. Amylases in combination with cellulases and pectinases are utilized during juice processing for maceration, liquefaction, and clarification, to improve yield and cost effectiveness[36,37].

Detergent Industry

In recent time, the use of enzymes in detergent formulations as additive has become inevitable due to milder conditions than the detergents containing chemicals[2,38]. This industry represents the leading utilization of industrial enzymes. The enzyme detergent formulations improve the stain removal effectiveness of the detergents in an eco-friendly manner[28]. Amylases catalyze the hydrolysis of glycosidic linkages in stains and eliminate the starchy glue that combine with other stains and dirt. The α-Amylase, an endo-amylase, is utilized primarily for laundry detergents, as exo-amylase activity is inefficient for stain removal. Currently, α-amylases are included in approximately 90% liquid detergent formulations[2,28,39,40]. The α-amylases from *Bacillus* and *Aspergillus* species exhibit perfect compatibility with detergent conditions and hence, these enzymes are added in laundry and dishwashing detergent formulations to remove residues of starch based food products such as cakes, chocolate, pasta, potato, gravies etc. The hydrolyzing activity of amylases also restrict binding of swollen starch to other stains and dirt[18,28,41]. Sometimes, a mixture of enzymes, including amylases, cellulases, pectinases, lipases and proteases is added in small quantities to improve effectiveness for stains removal and fabric care[1,2]. Amylases and proteases are added to dishwashing detergents for removal of spots containing food particles [42].The enzyme detergent formulations contain less bleaching agent, phosphates and therefore, have advantageous effects on human beings and environment[1,41,43].

Paper Industry

The utilization of microbial origin enzymes has developed progressively in paper and pulp industry due to increasing awareness of sustainability issues and to minimize unfavorable effects on the environment. The uses of enzymes in this industry diminish energy consumption, processing time and quantity of chemicals required for processing. The applications of amylases in this industry comprise coating of starch, de-inking, drainage improvement and cleaning of paper[1,44]. The primary application of α-amylase in paper industry is to produce high molecular weight starch with low viscosity through modification of starch of coated paper[32]. This process makes the smooth and strong paper to enhance the writing quality. α-Amylase is used to hydrolyze polymer partially in a batch or continuous process to sustain the starch viscosity for paper sizing. Paper sizing improve the quality of the finished product and improve the strength and stiffness in papers. Low temperature active α-amylases are of use to reduce starch viscosity for proper coating of paper[45,46,47].

Textile Industry

In this industry, amylases are exploited for desizing process i.e. removal of starch to improve uniform wet processing. Starch, low price and easily available sizing agent, is added to yarn for fast and secure weaving process. In textile weaving, starch paste is used for warping to gives strength and prevents loss of strings[48]. After weaving, amylase is added that specifically catalyze hydrolysis of starch to water soluble dextrins. Amylase efficiently removes starch without damaging the fabric[49]. The enzymatic desizing of cotton with α-amylases is state of-the-art since many decades[50]. Amylases from *Bacillus* strain are utilized in warp sizing of textile fibers[28].

Miscellaneous Applications

Besides amylases application in food, leather, alcohol, paper etc., spectrum of amylase uses has been expanded to many other fields. These are used in animal feed to enhance digestibility[51]. For analytical purposes, amylase based process used to detect oligosaccharides[2,52]. Amylase has also been reported for the production of gold nanoparticles[53]. In bioremediation, these enzymes are used to process starch containing wastes. Additionally, amylases are also used to split fibers in leather processing.

Conclusion

Enzyme based industries are gaining significance worldwide due to technical as well as biotechnological advantages. Enzyme mediated commercial processes are cost effective and environmental friendly. Amylases are mainly used in starch based food and non-food industries. Starch hydrolyzing activity of amylase is most widely applied in the production of fructose and glucose syrups and, in the starch processing prior to sugar fermentation by yeast for the production of ethanol. In food industries, they are used to prolong softness and shelf life of baked products. In textile and paper industry, these are utilized for removal of starch to improve uniform wet processing and, de-inking, improving paper cleaning and drainage improvement, respectively. With expanding biotechnological development, the application spectrum of amylases is also expanding in pharmaceutical and analytical applications.

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